



DEPARTMENT OF AGRICULTURE
BARBADOS.

RESEARCHES

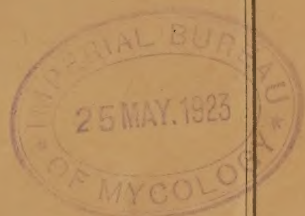
ON THE

ROOT DISEASE OF SUGAR CANE

BY

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Assistant Director of Agriculture,
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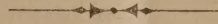


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B. A. BOURNE, B.Sc.

ASSISTANT DIRECTOR OF AGRICULTURE.

INTRODUCTION.

In view of the very great losses which have been attributed to "root disease" in the past and because the disease is without question one of the most serious ones affecting the sugar cane crop of this island at the present time, the writer has devoted a very considerable part of his time to field and laboratory work in this connexion. Besides, there is a great diversity of opinion at present as to the real cause or causes of that condition known as "root disease" and as long as this uncertainty exists there can be no real advances made towards eradicating or even controlling the disease in infested areas.

The very earliest observers on the nature of "root disease" have been of the opinion that several factors are involved, but one should not lose sight of the fact that even assuming that this view is correct there can be a possibility for considerable variation, not only in the number of factors existing, but also in the relative importance of the various factors from one country to another and even from one locality to another within the same country.

This paper is the outcome of extended research with a view of determining those possible factors contributing toward the "root disease" of sugar cane in Barbados and then applying them under controlled experimental conditions in order to establish their relationship to the disease.

HISTORICAL.

There has hardly been a plant disease in literature more subject to diversity of opinion and which has been under observation for a greater period of time by various workers without definite conclusions being arrived at, than that of the "root disease" of sugar cane. It was not that difficulty was experienced by the early investigators in associating some organism with the disease, but in every instance positive proof of the pathogenicity of the organism in question was lacking. Although the disease was apparently known to occur as far back as the middle of the 19th century, yet the first investigator to assign a definite cause to it seems to be Wakker^{18*} in the year 1895.

Inasmuch as the literature relating to past work on "root disease" until about the end of 1919 is so well summed up by Mr. F. S. Earle,⁷ of the Department of Agriculture, Porto Rico, the writer desires to refer readers to his recent publication in which he reviews briefly the work done approximately up to that date. For a detailed account of such past work readers may consult the literature by Wakker,¹⁸ Wakker and Went,¹⁹ Howard,⁹ Lewton-Brain,¹¹ Cook and Horne,⁵ Cobb,⁴ Fulton,³ Stockdale,¹⁷ Bancroft,¹ Johnston and Stevenson,¹⁰ Lyon¹² and Earle.⁷

Perhaps the greatest amount of light yet thrown on the problem of sugar cane root disease has been the result of the work of the last two years, particularly that of Mr. J. Matz, of the Department of Agriculture of Porto Rico. In his paper¹³ published in January 1920, he records the results of his researches with a view of establishing the parasitism of certain fungi (viz. *Rhizoctonia* and *Pythium*) and

†Forwarded to the Government for publication in August 1922.

*These numbers refer to the list of literature cited (see page 17).

their relation to root disease. Unfortunately, however, the fungi which he isolated from the roots of sugar cane and experimented with subsequently were not stated to be obtained from canes showing typical symptoms of root disease. Furthermore, in his main inoculation experiment the canes in all the pots grew normally so that the ability of the two fungi, viz., *Rhizoctonia* sp. and *Pythium* sp. isolated from cane roots to produce the symptoms of root disease was not demonstrated.

Peltier ¹⁶ has given a list of plants reported as susceptible to *Rhizoctonia solani* and it is seen therefrom that this fungus was isolated from roots of sugar cane in Louisiana, U.S.A., by Edgerton in 1912. The relation between this fungus and root disease of cane was not studied, however, and no inoculation experiments with this host are recorded by him.

Another equally important contribution to the solution of the root disease problem has recently been published by Mr. C. W. Carpenter, ³ Plant Pathologist of the Hawaii Federal Experiment Station. He has made a study of the so-called "deterioration" of the Lahaina (apparently the Bourbon variety) cane, still cultivated largely in those islands. Inoculation experiments in which a species of *Pythium* (which had been isolated from diseased roots) was used, proved quite successful. No details are given in regard to the external conditions which seem to favour the disease. It is significant too that the species of *Pythium* isolated from cane roots in Hawaii appears to be a much more vigorous parasite than the species found by Matz in Porto Rico. This difference in virulence, however, may in all probability be due mainly to the relative resistance of the various varieties in the two countries to the same fungus and perhaps to climatic conditions also.

In the light of the above researches therefore, it would appear as though the main factors contributing toward root disease may vary from one country to another. In Hawaii, *Pythium* sp. appears to be the virulent organism attacking cane roots, whereas in Porto Rico *Rhizoctonia* sp. predominates in virulence and activity as a root destroying agent while *Pythium* sp. does not take nearly as prominent a part in causing a pathological condition.

In January, 1920, Matz ¹³ described an organism found associated with basal internodes and roots of sugar canes which were attacked with top rot or rather dry top, a symptom often accompanying advanced stages of true root disease. It is stated that the above organism was found plugging the conducting vessels in the vascular system to varying extents, sometimes only three or four coloured bundles being plugged for about two or three inches through the lowest nodes and internodes of a cane stem while other stem bases were affected to a larger extent.

The writer regards the conclusions arrived at by Matz relative to this organism being a member of the family Plasmodiophoraceae as being entirely unjustified. All attempts at germinating the thick walled spores described failed and so the various stages in the life history which would place the organism in the family Plasmodiophoraceae or genus *Plasmodiophora* are entirely hypothetical. In view of the results of research into the causes of true root disease of cane in Barbadoe carried on by the present writer the question of the vascular bundle organism described by Matz will be discussed in another section (vide page 5).

There was also published by Matz ¹⁴ in January, 1921, a list of the various species of *Rhizoctonia* associated with diseased cane roots, viz. *R. solani*, *R. palida*, *R. ferruginea* and *R. grisea*. The cultural and other characteristics of these various species were given in detail together with their various host plants. No inoculation experiments with sugar cane were described, however, so that the ability of these various fungi to produce the true symptoms of root disease in sugar cane was not demonstrated.

SYMPTOMS.

In order to distinguish root disease from other pathological conditions which give cane plants a similar appearance in the field as in the case of the former disease, it is necessary to arrive at some suitable definition which would automatically segregate this particular disease from all others at present known to affect this host. At first sight, plants could readily be considered as being affected with root disease when other factors may actually be the cause of such a condition instead of those contributing towards true root disease as will be defined later. Matz has pointed out that in Porto Rico borers of various kinds, drought, lack of cultivation, gum disease, top rot and lack of drainage produce effects that may be taken for root

disease. Indeed, in Barbados the effect of an attack of the moth borer (*Diatraea saccharalis*, Fabr) or of the brown hard back (*Lachnosterna smithi*, Arrow) or of the root borer (*Diaprepes abbreviatus*, Linn) is sufficient to lead one astray in deciding whether root disease is attacking a certain stool or not unless careful investigations are made. The definition of true root disease of sugar cane as given by Matz, i.e., "a decomposition of roots taking place on account of the invasion of fungi", appears to be a satisfactory one from every point of view and causes any confusion which may have existed in the past with other cane diseases to disappear.

Plate 4, fig. 10* shows the roots and diseased condition of the base of the stem in a typical case of root disease. Most of the roots are seen to be dead while some of the white roots are dying and show distinct reddish areas at the points "a."

The primary symptoms of root disease therefore are decomposition and lack of healthy roots, stunted appearance of the cane, and an unhealthy yellowish green colour of the leaves which are often dried completely in advanced stages. This latter symptom, i.e., the yellowing of the leaves is considered a most constant phenomenon exhibited by all the varieties of cane examined by the writer, i.e., White Transparent, Ba. 6032, B.H. 10 (12) and others and has been reproduced repeatedly in his inoculation experiments. Because of the lack of healthy roots and the resultant impairing of the physiological functions, top rot may also occur. Furthermore, the shrivelling of the leaves during a hot day (see Plate 1 fig. 1, A) also suggests the inability of the roots to meet the needs of increased transpiration. The writer has confirmed the observations made by Matz relative to the absence of the fungus *Marasmius sacchari*, Wakker, binding the basal leaf sheaths to the stalk in otherwise typical cases of root disease. Indeed, in some instances other common saprophytic fungi, e.g., *Trichoderma lignorum* were present to the exclusion of *Marasmius*. Thus it is evident that in Barbados as in Porto Rico the presence of either one or both of these latter fungi commonly associated with decaying leaf sheaths and cane bases is of no significance whatever and may or may not be associated with typical cases of root disease, depending on whether they happen to form part of the fungus flora of the soil giving rise to root diseased plants. Plate 1 figs. 1 and 2 show typical fields of first ratoon canes of the variety B. H. 10 (12) which are attacked by root disease. Some plants are so seriously attacked (see fig. 2, B and C) that they are only about one half the size of those in their immediate vicinity (see fig. 2, D) which apparently have not yet contracted the disease but which did so a few weeks afterwards. The yellowish unhealthy appearance of the leaves of these attacked stools was very significant when a comparison was made with those plants which were not yet suffering from the disease although growing in the same field quite close to the former.

ECONOMIC IMPORTANCE AND DISTRIBUTION.

Without doubt root disease is of greater economic importance to the cane crop in Barbados at the present time than any other fungoid disease or insect pest. That it has been a serious factor in the economic production of cane sugar for some time past is evidenced by the numerous references made from time to time by various scientific workers. Bancroft¹ mentions that it was especially prominent in Barbados in 1904. South¹⁶ states that this disease was prevalent all over the island in 1910 and in some cases did a great deal of injury. Dash⁶ mentions that the root fungus was by far the most troublesome disease, particularly to young ratoon canes in the year 1916-17. The writer² called attention to the great economic loss to the island through root disease in 1920-21 and expressed the opinion that *Rhizoctonia* sp. which had been isolated from cane roots on more than one occasion during the year from typical cases of root disease, was mainly responsible for this loss.

ISOLATION OF FUNGI FROM ROOTS OF CANE SHOWING TYPICAL SYMPTOMS OF ROOT DISEASE.

The medium mostly used consisted of sterile sweet potato agar of the formula 150:10:500. The usual plate culture method was used throughout. Portions of diseased root about 8 mms. in length were first sterilized superficially by immersing in a disinfecting solution consisting of 50 c.c. of 1% HgCl_2 solution in H_2O + 50 c.c. of 50% alcohol (ethel) for from twenty to forty seconds. They were then washed

*Plates 1 to 5 photographs by author.

three times in sterile distilled water and transferred with a sterile needle to sterile petri plates, usually two portions to each plate of 10 cm. diameter. The melted medium being adjusted to a temperature of 40°--45° C. was now poured into each plate and rocked in the usual manner before setting down to harden. All operations of sterilizing, plating of roots, incubating and transferring, etc. were conducted in a special closed transferring and inoculating room in order to minimize chances for contamination by means of air currents. The plate cultures were incubated at room temperature and examined daily for any growth which may have started from the portions of root. As soon as any growth started from a root portion it was transferred as soon as possible to one or more slant cultures to isolate in pure culture and these culture tubes properly labelled with the origin of the cane root, type of medium and date. As soon as the particular fungus was identified the name was also added on the label in every case. Plate cultures were incubated until the root portions showed fungus growth or until accidental contamination occurred. If no growth appeared from a root portion after a month's incubation it was discarded. By following out these methods the various fungi associated with roots of cane plants suffering from root disease and showing typical symptoms of the same, were readily recovered in pure culture for future study and experimentation.

In selecting plants for experimental research an effort was made to obtain these from as many different parts of the island representing different soil types as possible. Only plants which showed typical symptoms of root disease were made the subject of detailed examination and study.

RESULTS OF ISOLATION OF ORGANISMS ASSOCIATED WITH ROOTS AND STEM BASES OF CANES SHOWING TYPICAL SYMPTOMS OF ROOT DISEASE.

Attention was at first directed to the fungi associated with the diseased adventitious roots. The first cases of root diseased canes studied were those of first ratoons of the varieties B. H. 10 (12) and Ba. 6032 on a plantation in the parish of St. John. A species of *Rhizoctonia* having cultural and other characteristics of *Rhizoctonia solani*, Kuhn was obtained in pure culture from diseased roots of both varieties in several instances from these particular fields. In very few instances were the fructifications of *Marasmius sacchari* seen at the base of root diseased stools in these fields and in no instance was it isolated from dying roots. Similar cases of root diseased canes of the same varieties and of first ratoons were examined at various plantations in the parish of St. Michael and in every instance examined a species of *Rhizoctonia* was recovered in pure culture. At one plantation a species of *Rhizoctonia* having cultural characteristics resembling those of *Rhizoctonia palida* described by Matz was recovered in pure culture. Of the *Rhizoctonias* only this latter species was recovered from this particular field in spite of numerous attempts to obtain other forms of this genus. In the parish of St. Joseph a field of fourth ratoon canes of the variety White Transparent was reported to be suffering from root disease about the middle of July 1921. The writer visited the field in question and found most of the stools in the field to have all the typical symptoms of root disease. The pronounced uniform yellowing of the leaves throughout the whole field coupled with the stunted appearance of the stools, presented a picture not easily to be forgotten. Numerous isolation trials were made from diseased and dying roots and in several instances *Rhizoctonia solani* was recovered in pure culture. A few weeks later, more isolation trials were made from further specimens taken from other parts of the same field and the same fungus was obtained in pure culture as before. In some instances, however, on both occasions a species of *Fusarium* developed from diseased roots and was isolated in pure culture. The occurrence of *Fusarium* sp. as associated with the root disease of sugar-cane aroused particular interest, since it had never been recorded before in literature to the knowledge of the writer.

While carrying out isolation trials from the roots of root diseased canes from various parishes it was observed that in practically every instance where dead cane roots were plated either *Trichoderma lignorum* or *Marasmius sacchari* or both were recovered in pure culture. It was also observed that in no instance was either of these fungi recovered from freshly diseased portions of roots. These were very significant facts and threw considerable light on the question of the primary cause or causes of root decay, even before any inoculation experiments were conducted with pure cultures.

In the course of examining root diseased stools from various parishes it was observed that very often the internal tissue of the basal portions of stems although having no insect injury or wound was dirty reddish in colour (see Plate 4 fig 10, b.) Such a condition was more readily found in the case of advanced stages of the disease. Several attempts were made at isolating the fungi associated with this dirty reddish tissue found at the basal portions of stems. The same technic was used in the isolation trials here as in the case of adventitious roots. As a result of these isolation trials it was found that either *Rhizoctonia solani* or *Rhizoctonia palida* was present. In some cases in addition to one or other of the above fungi either *Fusarium* sp. or *Trichoderma lignorum* or *Marasmius sacchari* or combinations of these three latter fungi were also present. All of these fungi were recovered in pure culture and they agreed in every respect with the pure cultures of the various species obtained from the adventitious roots.

While making an examination of the basal portions of a root diseased stool a typical reddening of the fibro-vascular bundles was found to be present which appeared to resemble that condition described by Matz¹⁵ as due to *Plasmodiophora vascularum* n. sp. Portions of the diseased base were accordingly embedded in paraffin and the tissues made the subject of histological investigation. Some of this same tissue untreated was removed with a sterile knife and cut into fairly thin slices and mounted in sterile distilled water on microscopic slides with cover glasses and placed in a sterile damp chamber. After twenty-four hours, the sections were examined microscopically and the liquid surrounding the sections was found to be practically alive with very active ciliate protozoan organisms. There were also present numerous encysted forms of these protozoa and the writer was at once struck with the remarkable resemblance between these cysts and the spherical, smooth, evenly or sometimes coarsely granulated spores with thick hyaline walls described by Matz. At first these cysts were almost hyaline to greyish in colour but on keeping the slides for several days orange yellow to brownish cysts were formed in abundance. It is remarkable too that these yellowish or brownish cysts have never been seen to germinate, a phenomenon also characteristic of the organism described by Matz. Measurements of these mature orange yellow to brown cysts showed them to vary mostly from 13.5-16 microns in diameter, but variations were found ranging from 8-19 microns in diameter. Examination of a series of microtomic sections of the basal tissue of the same cane showed that the fibro-vascular bundles in some instances contained what appeared to be the thick walled cysts as were seen on the slides mentioned above. In some cases where the vessels were plugged, the distinct characteristic kidney shaped forms of the active, non-encysted protozoan organism could be seen. It may be mentioned here that the adventitious roots of the cane stool from which this organism was first obtained were found to be seriously attacked by the fungus *Rhizoctonia solani*, Kuhn. Since then other cane stools whose adventitious root systems were found to be attacked by one of the root destroying fungi and whose basal stem portions showed signs of decay, have been found to be infested with the above protozoan organism to varying extents in the lowest internodes. (For description of protozoan organism see Appendix page 17)

FIELD CONDITIONS ASSOCIATED WITH CASES OF ROOT DISEASE.

At this point it would appear advisable to note more particularly the cultural treatment and history of those fields of canes from which typical cases of root disease were taken for detailed examination and study of their root systems. Indeed, from a consideration of this phase of the question the writer hoped to discover some of the main factors which seem to favour the disease.

One of the main points which the writer noted when he visited the various fields affected by root disease was that none of them were covered with trash as is usually done with young plant canes. None of the fields in question had first crop canes but the age of the plants varied from first to fourth ratoons. Another point noted was that in every instance the fields had experienced an extremely dry period at some time just before the majority of the plants were reported to be looking stunted. These are very significant facts and will be discussed later in connexion with the relation between the parasites and host in the light of extended research carried out since 1912 by Mr. G. L. Peltier,¹⁶ of the Agricultural Experiment Station, University of Illinois.

ETIOLOGY OF THE DISEASE.

(a) Formal Proof of Pathogenicity of:—

1. *Rhizoctonia solani* and
2. *Rhizoctonia palida*.

There is no doubt that root disease is due to one or the other of the above fungi as determined by the following points:—

1. The constant association of one or the other of the above fungi with the disease and their isolation from typical diseased tissue of the host.
2. Healthy sterilized cuttings when inoculated with pure cultures of either of the above fungi in sterilized soil produced plants having characteristic signs of the disease. The penetration of the fungi into the roots has also been observed.
3. The fungi have been re-isolated from inoculated diseased roots and when compared in culture with the fungi used for inoculation were identical with them.

(b) Details of Inoculation Experiments.

Experiment 1. Rhizoctonia solani.

Eight cuttings of Ba. 6032 variety consisting of one entire internode and two nodes in each case were selected from a healthy plant, sterilized by immersing in an aqueous solution of mercuric bichloride (1-1000) for 15 minutes and then rinsed in sterile distilled water and planted in sterilized soil which had been heated in the autoclave at 120° C for 15 minutes. The soil was placed in large sterilized glass petri dishes and one cutting planted in each dish. Six cuttings were now inoculated by placing the contents of a tube of a pure culture of *R. solani* in contact with the nodes (just where the roots arise) in each case and covering them over with soil. Sterile distilled water was now added to make the soil damp and the covers replaced. The other two control cuttings were treated in a similar manner but no culture was added. The dishes with the cuttings were placed outside every day so that the warmth from the sunlight should duplicate that obtained by cuttings under field conditions. After six days all the cuttings were examined and it was found that germination had taken place in every case. In every single case several of the roots arising from the inoculated cuttings were reddish in colour and diseased looking while those of the controls were white and healthy looking. Several portions of diseased root from each inoculated cane were now plated out on sterile medium in the usual manner after sterilizing them superficially and from the roots of five different cuttings the fungus was recovered in pure culture. The portions from the other inoculated cutting remained in culture for nearly a month without developing any growth. The fungus recovered in pure culture was identical with that used for inoculum in every instance. Sections of the diseased tissue also showed the fungus to be present therein.

Experiment 2. Rhizoctonia palida.

Carried out exactly as in experiment 1 above except that two inoculated and two control cuttings were used. In both cases the identical fungus as was used for inoculum was recovered in pure culture from the diseased root portions of the inoculated cuttings while the controls remained normal. When sections of the diseased tissue were examined the fungus could be seen to be present therein.

Experiment 3. Marasmius sacchari.

Carried out exactly as in experiment 1, except that two inoculated and two control cuttings were used. In no instance did the roots of the inoculated cuttings show a pathological condition. Plate cultures were made of various portions of roots which had been in contact with the fungus but this latter was not recovered in pure culture in a single instance.

Experiment 4. Fusarium sp.

Carried out exactly as in case of experiment 1, except that two inoculated and two control cuttings were used. Results were the same as in the case of experiment 3.

Experiment 5. Rhizoctonia solani.

Four equal sized cuttings of the variety Ba. 6032 consisting of one internode and two nodes in each case were selected from the upper part of a healthy plant, sterilized and washed as in experiment 1, and each planted separately in soil (sterilized in the autoclave as in experiment 1) placed in five gallon kerosene tins which were previously scoured out and rinsed with boiling water. Typical black limestone soil was used having very good texture and was thoroughly mixed to ensure uniformity of soil conditions in all cases. Ample drainage openings were provided in the bottom of the tins and a layer of sterilized drainage stone placed in the bottom before filling up with sterilized soil to within two inches of the top in each case. Two of the cuttings were inoculated by placing on each the contents of a culture tube containing a pure culture of *R. solani* and then covering them over with sterile soil. The other two were left as controls and all the tins were then placed outside in a position where all the plants would receive equal amounts of sunlight and watered with tap water when required. Occasionally, watering was stopped for a short period so as to imitate a period of drought. This experiment was started on July 2, 1921.

As will be seen from plate 3 fig. 5, there was no visible difference in growth of any of the plants fifty-six days after the planting of the seed pieces. Shortly after this however, there was a gradual yellowing of the leaves of the inoculated plants, Nos. 2 and 3, when compared with those of the controls Nos. 4 and 5 which were normally green. On February 16, 1922, there was very pronounced yellowing of the leaves of the inoculated plants when compared with the controls although there was no visible difference in growth as before, and on this day all the shoots of the plants both inoculated and controls were cut off. Each plant was cut off with a knife, the blade of which was first placed in boiling water for several minutes and then allowed to cool.

Three weeks after the shoots of all the plants had been cut off, (i.e., on March 9, 1922) a photograph of the plants was taken (see Plate 3 fig 6). It will be seen, therefrom that the rate of growth is distinctly in favour of both controls: Nos. 4 and 5.

On April 13 1922, another photograph (see Plate 3 fig. 7) was taken of all the plants in order to show the amount of growth which took place in the inoculated and control plants subsequent to the photo taken on March 9. The yellow appearance of the leaves, and stunted condition of the inoculated plants Nos. 2 and 3 when compared with the normal green foliage and healthy growth of the controls Nos. 4 and 5 furnished ample proof of the ability of *Rhizoctonia solani* to reproduce the symptoms of root disease.

Plate 4 fig. 8 shows a photograph of the root systems of the plants in the above experiment. The photo was taken on April 21, 1922. The amount of roots of the control plants Nos. 4 and 5 are seen to be somewhat greater than those of the inoculated plants particularly No. 2. Furthermore, there was hardly to be found a living root in the inoculated plants while healthy white roots were abundant in the controls.

That the amount of root system associated with seedling cane plants is apparently also influenced by the inoculation of the soil with a pure culture of *R. solani* is also shown by the experiment now to be described.

Experiment 6. Rhizoctonia solani.

Two eight inch pots containing sterilized soil (sterilized in autoclave as in the above experiments) were planted to young cane seedlings of the variety B. 60. The seedlings were removed from their original pots and the soil adhering to the roots washed off in tap water before replanting. One of the pots was inoculated with a pure culture of the above fungus while the other was left as a control. Although conditions were not such as to exclude the possibility of the control being contaminated by *Rhizoctonia* which may have been present in the soil of the original pot when the young seedling was transferred, yet equal conditions in this respect obtained for both inoculated and control plants and so the experiment is not without value. Seedlings of equal age, size, and healthy appearance were selected for the experiment.

The results of this experiment are shown in Plate 4 fig. 9. No. 1 is the inoculated plant and No. 2 the control. The photograph was taken thirty-four weeks after the seedlings were transplanted and inoculation done. Not only is there a greater amount of root system in the control plant but the amount of stem growth is greater.

Experiment 7. *Rhizoctonia palida*.

This was carried out exactly as in the case of experiment 5 using the variety Ba. 6032 as before. The planting and inoculation of the cuttings were done on February 15, 1922.

Plate 5, fig. 11 shows a photograph of the inoculated and control plants when sixty-five days old. Nos. 1 and 2 are the inoculated and Nos. 3 and 4 the controls. There can be seen no apparent difference in growth as yet between any of the plants. At this stage the colour of the leaves in every case was normally green. The plants were cut off on the same day this photograph was taken, i.e. on April 21.

Subsequent to the plants being cut off on April 21 sprouting occurred normally in every case. After a few weeks' growth, however, the foliage of the inoculated plants Nos. 1 and 2 gradually showed a yellow appearance when compared with the controls Nos 3 and 4 and on June 7, when the total age of the plants was now 112 days, this yellowing of the leaves in the case of the inoculated plants was very pronounced when compared with the normal green foliage of the controls. If watering was discontinued for a few days the leaves of the inoculated plants at this stage also showed serious shrivelling during the middle of a hot day while the controls under exactly the same conditions showed no such symptom of disease.

On July 21, a photograph was taken of all the plants in the experiment (see plate 5 fig. 12). At this stage the total age of the plants was 156 days and symptoms of stunting were already evident in the case of the inoculated plants, particularly No 1. when compared with the controls. As in the case of experiments with *Rhizoctonia solani*, the pronounced yellowing of the leaves and symptoms of stunting in the case of the inoculated plants when compared with the normal green foliage and healthy growth of the controls furnished abundant proof of the ability of *Rhizoctonia palida* to reproduce the typical symptoms of root disease. An examination was also made of the root system of all the plants as in Experiment 5 and it was found that the inoculated ones were much decayed and it was difficult to find a living healthy root whereas both controls possessed an abundance of healthy roots.

THE RELATION OF *R. SOLANI* AND *R. PALIDA* TO THE SUGAR CANE.

Although a complete survey as regards these two species of fungi has not been made, yet every indication exists that either one or the other of them and probably both are to be found throughout the island. Peltier¹⁵ states that *Rhizoctonia* is apparently universally present in the soil where it can live indefinitely on dead organic matter under ordinary conditions thus making it a dangerous fungus.

Matz¹⁴ in Porto Rico has found *R. palida* attacking the roots of pepper (*Capsicum* sp.) and corn seedlings (*Zea mays*). He has also isolated *R. solani* from partially decayed bean pods touching the ground, from citrus (Grapefruit seedling in pot), Roselle (*Hibiscus sabdariffa*) seedling, celery, tomato stem, lettuce leaves, sugar cane roots, banana roots and pea. This latter fungus is stated by Peltier¹⁵ to attack some 165 species of plants in the United States of America including all the more important families of dicotyledons, a number of monocotyledons, several gymnosperms and *Equisetum*. Most of the floricultural plants, vegetable and field crops, herbaceous plants and many weeds are reported to be susceptible to the attacks of this fungus.

Researches carried out both by Peltier in the United States of America and Matz in Porto Rico have shown, therefore, that the above two fungi apparently show no marked specialization of host. This is a very significant fact and as pointed out by Peltier with reference to *R. solani* these various hosts assist in harbouring the fungus and in keeping up its virulence. The same of course holds true with regard to *R. palida*.

The ability of these sclerotia producing fungi to withstand adverse conditions is another most important point for consideration. Peltier¹⁵ experimenting with *R. solani* found that soil cultures started in December 1911 kept in the laboratory and allowed to dry out, yielded pure cultures as late as July, 1914. No such experiment with *R. palida* is recorded in literature but it is very probable that similar powers of resistance would be exhibited by this fungus also as indicated by the nature of its sclerotia. In the absence of a host plant and under adverse moisture conditions, therefore, the eradication of these fungi seems to be a matter of impossibility. Such conditions however would no doubt be extremely beneficial in reducing the virulence of the organisms so that plants grown subsequently in that soil would not be so subject to attack.

In summing up the results of his researches on Parasitic *Rhizoctonias* in America, Peltier¹⁵ states: "A certain vigor of mycelium must be attained before *R. solani* is able to attack a plant. A high temperature (88° F.) together with either too little or too much moisture, determines to a large degree the virulence of the strains. It is only under certain conditions that the fungus becomes a dangerous parasite." There seems therefore to be a distinct correlation between the conditions which existed in fields suffering from root disease and those found by Peltier¹⁵ to favour the parasitism of *R. solani*. The untrashed fields were no doubt subject to quite high temperatures for the soil in the kerosene tins during the inoculation experiments with the two species of *Rhizoctonia* which was left untrashed was tested and it was found that on a normal sunny day in June the soil which had been thoroughly watered at 8 a.m. reached a temperature of 95.0° F. at 3 p.m. three inches below the surface. The high temperature of the soil in the fields coupled with drought conditions evidently soon brought about a very low soil moisture content thus lowering the vitality of the plants and producing those conditions which favour the virulence and parasitism of the above fungi.

The fact that plant canes are apparently not found attacked by root disease to a noticeable extent is very significant. Even in the inoculation experiments with the two species of *Rhizoctonia* the typical symptoms of root disease did not appear immediately upon the germination of the cane seeds although an ample amount of the organism was present in each case. This suggests that after these organisms have been forced to lead a saprophytic life such as exists when grown in artificial cultures or in fields which have been thrown out of cultivation, they lose their parasitic properties to such an extent that they are unable to attack healthy roots until conditions of too little or too much moisture and a high temperature so lower the vitality of the roots and favour the vitality of the fungi as to allow the latter to become parasitic. Under favourable conditions these fungi evidently may become very virulent especially if sugar cane or any other of their hosts are grown continuously on the same soil for a long period. It is indeed a well known biological phenomenon that the virulence of a parasite can usually be greatly increased by a series of inoculations upon its host successively. There can be no doubt that but for the fact that cane fields are occasionally "thrown out" in this island for a period of about six months, serious epidemics of this disease would often occur.

The question of the relative resistance of the various varieties of sugar cane to the disease is a point of very great economic importance and worthy of intensive study. Indeed, by the crossing of resistant varieties and selection it may be possible to develop varieties entirely immune to the disease under ordinary adverse conditions.

THE MORPHOLOGY IN CULTURE OF THE FUNGI CAUSING ROOT DISEASE.

The morphological characteristics of both *Rhizoctonia solani* and *Rhizoctonia palida* have been so well described and figured by Matz¹⁴ that there is no need to add anything further in this connexion. Plate 2 fig. 3 and 4 shows photographs of the above two fungi in culture flask and tube and illustrates their chief distinguishing characteristics. The culture of *R. solani* in the flask of sweet potato chips is 123 days old while that in the tube of ash maltose agar is ten days old. The culture of *R. palida* in the flask of sweet potato chips is fifty-four days old and that in the tube of sweet potato agar, ten days old.

GROWTH ON DIFFERENT MEDIA OF THE FUNGI CAUSING ROOT DISEASE.

Details of the growth of *R. solani* and *R. palida* on all the media are given below. A comparative study of the growth of these fungi has been made on ten different media. Two tubes of each medium were inoculated on the same day and all these tubes were kept at room temperature. All the media used were acid in reaction, ranging from +3° to +20° Fuller's scale. The composition of the media is given in Appendix (pages 15 and 16).

A. *Rhizoctonia solani*.

1. Sweet Potato Agar. (+6° Fuller's scale)

Growth after seventy-two hours covers $1\frac{1}{2}$ " in length of surface of medium; aerial, fine, transparent, much branched; mycelium climbing walls of tube to fair extent. Diameter of mycelium 4-8 microns.

After five days mycelium covering walls of tube completely with abundant formation of dirty yellowish sclerotia thereon. Good aerial growth which is beginning to form aerial sclerotia. Ranks second in rate of growth on media tried.

After fourteen days good production of brown sclerotia on walls of tube as well as many white to brown aerial sclerotia. Mycelium sometimes attaining a diameter of 13.5 microns.

2. Corn Meal Agar. (+3° Ful).

Growth after seventy-two hours covers $1\frac{7}{8}$ " of the length of the surface of the medium; aerial, fine, transparent, much branched; mycelium climbing walls of tube to fair extent. Diameter of mycelium:—4-8 microns.

After five days growth only fair production of aerial mycelium on which minute sclerotia are beginning to form. Only slight production of sclerotia on walls of tube. Rate of growth ranks fourth among media tried.

After fourteen days only a few small dark brown sclerotia on walls of tube with fair production of yellowish brown aerial sclerotia at upper extremity of medium. Diameter of mycelium reaching 12 microns.

3. Cane Stem Infusion Agar (+5° Ful)

Growth after seventy-two hours covers 1.0" in length of surface of medium; flat, poorly branched, fine, transparent and hardly visible superficially. A few strands of the mycelium climbing walls of tube. Diameter of mycelium:—3-6 microns.

After five days growth mycelium covering surface of medium. Very slight production of sclerotia on walls of tube; aerial mycelium poor. Ranks fourth among media tried in rate of growth.

After fourteen days fair production of small brownish sclerotia on walls of tube. Slight production of dirty white aerial sclerotia at upper extremity of medium. Diameter of mycelium reaching 13.5 microns.

4. Soil Infusion Agar. (+4° Ful)

Growth after seventy-two hours covers $2\frac{1}{2}$ " in length of the surface of the medium; flat, but somewhat aerial towards the extremity of the growth, fine, transparent and fairly well branched. Fair growth on walls of tube. Diameter of mycelium:—4-8 microns.

After five days growth, only slight production of aerial mycelium and sclerotia. Good production of minute brownish sclerotia on walls of tube. Ranks fourth in rate of growth among media tried.

After fourteen days sclerotia on walls of tube dark brown but no larger than before. Diameter of mycelium reaching 16 microns.

5. Ash Maltose Agar. (+6° Ful)

Growth after seventy-two hours covers $1\frac{1}{2}$ " in length of surface of medium,

fine, transparent and somewhat aerial particularly toward the extremity of the growth. Fairly well branched, mycelium climbing walls of tube to great extent. Diameter of mycelium :—4-8 microns.

After five days growth, only fair production of aerial mycelium and sclerotia. Good production of yellowish brown sclerotia on walls of tube. Ranks third among media tried in rate of growth.

After fourteen days, sclerotia on walls of tube quite large, often 2 mms. diameter and very dark brown. Aerial sclerotia white to brownish and small, only produced at upper extremity of medium. (see photograph Plate 2 fig. 4. B.) Diameter of mycelium :—13.5 microns.

6. *Lachnosterna grub* Agar (+9° Ful)

Growth after seventy-two hours covers $1\frac{9}{16}$ " length of surface of medium; fine, flat, transparent, poorly branched and hardly visible superficially. Few strands of mycelium climbing walls of tube. Diameter of mycelium :—3-7 microns.

After five days, growth scarcely visible. No aerial mycelium or sclerotia and no sclerotia on walls of tube. Ranks sixth in rate of growth among media tried, being easily the worst of all.

After fourteen days, no further growth than above.

7. *Diaprepes grub* Agar (+20° Ful)

Growth after seventy-two hours covers $1\frac{1}{2}$ " of the length of the surface of the medium; aerial, fine, transparent and much branched. Mycelium almost covering walls of tube. Diameter of mycelium :—4-8 microns.

After five days growth, abundant production of aerial mycelium completely filling tube. Very good production of aerial sclerotia on walls of tube. Ranks first in rate of growth among media tried.

After fourteen days, good production of very minute yellowish to dark brown sclerotia on walls of tube with good production of aerial sclerotia also. Mycelium sometimes attaining diameter of 13.5 microns.

8. *P. Beef* Agar. (+10° Ful)

Growth after seventy-two hours covers 2.0" in length of surface of medium; flat for most part but slightly aerial toward extremity of growth; fine and transparent. Good growth on walls of tube. Diameter of mycelium :—4-8 microns.

After five days, fair production of aerial mycelium with practically no aerial sclerotia. Fair production of minute sclerotia on walls of tube particularly toward base. Rate of growth ranks fourth among media tried.

After fourteen days, little further additional sclerotial development. Mycelium reaching 11 microns in diameter.

9. *Glucose P. Beef* Agar. (+10° Ful).

Growth after seventy-two hours covers 2.0" in length of surface of medium; flat for most part but slightly aerial toward extremity of growth; fine and transparent. Small growths on walls of tube. Diameter of mycelium :—4-8 microns.

After five days, good aerial growth and production of aerial sclerotia as well as sclerotia on walls of tube. Ranks second in rate of growth among media tried.

After fourteen days, aerial sclerotia production one of best on media tried. Little further development of sclerotia on walls of tube. Aerial sclerotia yellowish white to brown. Diameter of mycelium sometimes 13.5 microns.

10. *Glycerine P. Beef* Agar. (+10° Ful).

Growth after seventy-two hours covers $1\frac{1}{2}$ " in length of surface of medium; flat

and scarcely visible except for aerial portion toward extremity of growth; fine and transparent. Little growth on walls of tube. Diameter of mycelium:—3-7 microns.

After five days, upper surface of medium not covered, but a very good production of aerial growth and aerial sclerotia. Fair production of sclerotia on walls of tube. Ranks second in rate of growth among media tried.

After fourteen days, aerial sclerotia production one of best among media tried. Sclerotia dirty white to brown. Sclerotia on walls of tube often quite large and sometimes reach 2 mms. diameter. Diameter of mycelium reaching 13.5 microns.

B. *Rhizoctonia palda*.

1. *Sweet Potato Agar*. (+6° Ful.)

Growth in forty-eight hours covers $1\frac{3}{4}$ " in length of tube culture, the mycelium climbing the walls of the tube. Growth fine, transparent, plainly visible and flat. Diameter of mycelium:—4-7 microns.

On fourth day, pale yellowish-white, flat sclerotial mass formed on sides of tube particularly near base, almost meeting in centre, and also on surface of medium toward upper extremity. Sclerotial formation one of best of all the media tried. The rate of growth ranks first on media tried.

On thirteenth day, no great change from above except that the sclerotial mass is more dense and inclined to be leathery and extends further on the walls of the tube. Diameter of mycelium reaches 13.5 microns.

2. *Corn Meal Agar*. (+3° Ful.)

Growth in forty-eight hours covers $2\frac{1}{8}$ " of the length of the tube culture, the mycelium climbing the walls of the tube and almost meeting from both sides. Growth fine, transparent, flat and plainly visible. Diameter of mycelium:—4-8 microns.

On fourth day, pale yellowish-white, flat sclerotial mass beginning to form on walls of tube near base, and also along edge of medium. Ranks third in rate of growth on media tried.

On thirteenth day, sclerotial mass not extending much further, but somewhat denser. Not leathery. Mycelium reaching 13.5 microns in diameter.

3. *Cane Stem Infusion Agar*. (+5° Ful.)

Growth after forty-eight hours covers $2\frac{1}{8}$ " of the length of the tube culture, the mycelium climbing the walls of the tube and almost meeting from both sides; growth fine, transparent, plainly visible and flat. Diameter of mycelium:—4-7 microns.

On fourth day, yellowish-white sclerotial mass now beginning to form along edge of base of the medium and to some extent along the sides also. Along with corn meal agar culture which it equals in rate of growth, it ranks third in rate of growth of all the media tried.

On thirteenth day, sclerotial mass not extending much further but somewhat more dense. Not leathery. Mycelium reaching 16 microns in diameter.

4. *Soil Infusion Agar*. (+4° Ful.)

Growth after forty-eight hours covers $1\frac{1}{2}$ " of length of tube, the mycelium climbing the walls of the tube to a slight extent only; very fine growth and hardly visible it is so transparent and flat. Diameter of mycelium:—3-7 microns.

On fourth day, all of surface of medium covered but no sclerotial formation visible. Growth is one of worst now of media tried and ranks seventh.

On thirteenth day, no further change observed in amount of growth. No sclerotia formed. Diameter of mycelium reaching 13.5 microns.

5. *Ash Maltose Agar* (+6° Ful).

Growth in forty-eight hours covers $1\frac{5}{8}$ " of length of tube, mycelium climbing the walls of the tube and almost meeting from both sides; very fine growth and so transparent and flat as to be hardly visible. Diameter of mycelium :—4–7 microns.

On fourth day, good formation of yellowish-white sclerotial mass on walls of tube at base and to slight extent along edge of medium higher up. Slight aerial growth at upper extremity. Ranks fourth in rate of growth on media tried.

On thirteenth day, scarcely any further growth observed except that sclerotial mass is somewhat more dense and inclined to be leathery. Diameter of mycelium reaching 16·2 microns.

6. *Lachnosterna grub Agar*. (+9° Ful).

Growth after forty-eight hours covers $1\frac{3}{8}$ " of the length of the tube of medium; mycelium sparsely branched and only climbing the walls of the tube to a slight extent; growth so very fine, poorly branched, transparent and flat as to be scarcely visible. Diameter of mycelium :—3–7 microns.

On fourth day, all of surface of medium covered but no sclerotial formation visible. Growth is one of worst of all media tried and along with soil infusion agar, it ranks seventh.

On thirteenth day, no further growth or sclerotial formation. Diameter of mycelium reaches 11·0 microns.

7. *Diaprepes grub Agar*. (+20° Ful).

Growth after forty-eight hours covers one inch in length of the surface of the medium; mycelium much branched and climbs walls of tube to fairly good extent; very fine growth, whitish in colour; flat and plainly visible superficially. Diameter of mycelium :—4–8 microns.

On fourth day, small yellowish-white sclerotia present at edge of medium particularly at base and on walls of tube opposite medium. Quite a little white aerial growth on surface of medium. Rate of growth ranks second on media tried.

On thirteenth day, dirty whitish sclerotia on walls of tube, up to one millimeter in diameter, often coalescing. Coalesced masses somewhat leathery. Mycelium reaching 13·5 microns in diameter.

8. *P. Beef Agar* (+10° Ful).

Growth after forty-eight hours covers $1\frac{5}{8}$ " of the length of surface of the medium; very fine mycelium, much branched; hyaline, flat and hardly visible superficially, growth on walls of tube fair. Diameter of mycelium :—4·7 microns.

On fourth day, all of surface of medium not yet covered but minute yellowish-white sclerotia beginning to form to very slight extent at edge of same. Slight aerial growth also present on surface of medium. Rate of growth ranks sixth among media tried.

On thirteenth day, whole surface not yet covered. Sclerotia very few, yellowish-white and minute, often coalescing. Coalesced masses not leathery. Diameter of mycelium reaching 11 microns.

9. *Glucose P. Beef Agar*. (+10° Ful).

Growth after forty-eight hours covers $1\frac{1}{4}$ " of length of surface of medium, very fine, good growth on walls of tube, mycelium much branched but so hyaline and flat as to be scarcely visible. Diameter of mycelium :—4·7 microns.

On fourth day, yellowish-white sclerotia starting along edge of medium particularly toward the base and also on walls of tube opposite medium. Quite a little white aerial growth on surface of medium. Rate of growth ranks second on media tried. Diameter of mycelium reaching 13·5 microns.

On thirteenth day, dirty white minute sclerotia on walls of tube of irregular shape, often coalescing. Coalesced masses quite leathery. Best growth at upper extremity of medium of all media tried.

10. *Glycerine P. Beef Agar.* (+ 10° Ful).

Growth after forty-eight hours covers $\frac{7}{8}$ " of length of surface of medium. Very fine. No growth on walls of tube; mycelium much branched, flat, hyaline and scarcely visible superficially. Diameter of mycelium: - 4-7 microns.

On fourth day, all of surface of medium not yet covered but yellowish-white sclerotial formation present to fair extent at edge of medium and on walls of tube. Quite a fair aerial growth on surface of medium. Ranks third in rate of growth on media tried.

On thirteenth day, aerial growth quite thick with production of minute whitish aerial sclerotia. Sclerotial masses at edge of medium and walls of tube, yellowish to brown, thick and leathery. Diameter of mycelium reaches 13.5 microns.

THE EFFECT OF THE REACTION OF THE MEDIUM ON THE FUNGI CAUSING ROOT DISEASE.

These experiments were conducted in duplicate using glucose peptone beef agar in every case. Twenty tubes were inoculated in each experiment, two of each reaction, ranging from - 15° to + 30° Fuller's scale.*

1. *Rhizoctonia solani*, Kuhn.

This fungus was not affected by the reaction of the medium between the above ranges. The growth in every case after twelve days was considered very good.

2. *Rhizoctonia palida*.

Tube No.	Reaction of medium in degrees Fuller's scale.	Growth after 12 days.	Remarks
1	- 15	Fair	Aerial mycelium fair.
2	- 10	Fair	" " "
3	- 5	Fair	" " "
4	0	Fair	" " "
5	+ 5	Good	Aerial mycelium good.
6	+ 10	Good	" " "
7	+ 15	Good	" " "
8	+ 20	Good	" " "
9	+ 25	Very good	Aerial mycelium filling tube.
10	+ 30	Very good	" " "

SUMMARY.

1. Root disease is not confined to any specific type of soil, but occurs both on typical black as well as red soils.

2. Under the existing agricultural methods plant canes do not appear to be attacked by the disease to a noticeable extent, but ratoon canes seem to be especially susceptible under certain conditions.

3. Either *Rhizoctonia solani* or *Rhizoctonia palida* has been found associated with freshly diseased and dying cane roots in typical cases of root disease. In advanced stages of the disease the tissues of the basal portions of the stem are also infested by one or the other of these fungi. Sometimes, in addition to one of the above fungi *Fusarium* sp. is also found.

*Media from + 15° to + 30° Fuller's scale remained in the liquid condition on account of too much acidity.

4. *Marasmius sacchari* has never been isolated from freshly diseased and dying cane roots, but only from dead ones.

5. Inoculation experiments have proved conclusively that *Rhizoctonia solani* and *Rhizoctonia palida* are both parasitic and each can cause root decay and reproduce the typical symptoms of root disease, including stunting of the plant and yellowing of the leaves, while control plants under the same conditions show no such pathological symptoms.

6. Inoculation experiments with both *Marasmius sacchari* and *Fusarium* sp. have given negative results.

7. The reaction of the medium between -15° and $+30^{\circ}$ Fuller's scale has no effect on the growth of *Rhizoctonia solani*. On the other hand *Rhizoctonia palida* is affected by the reaction of the medium between these two ranges and grows best between $+25^{\circ}$ and $+30^{\circ}$ Fuller's scale with comparatively good growth between $+5^{\circ}$ and $+20^{\circ}$ Fuller's scale and fair growth between -15° and 0° Fuller's scale. Both fungi, however, appear to be adapted to quite wide ranges in medium reaction.

8. Both *Rhizoctonia solani* and *Rhizoctonia palida* are cultivable on most artificial media, but of those tried they give the best growth on Diaprepes grub agar, glucose peptone beef agar, glycerine peptone beef agar, sweet potato agar, and corn meal agar.

9. Conditions of high soil temperature combined with lack of moisture appear to favour the disease in the field.

RECOMMENDATIONS.

1. The planting of varieties found to be resistant to the disease.
2. The planting of healthy cuttings from plants which are free from the disease.
3. The suitable rotation of crops so that the parasitic root fungi are forced to live saprophytically thus reducing their virulence.
4. Proper tillage and drainage so as to provide good soil aeration and "root room" thus aiding the plants to maintain their maximum vitality.
5. The proper trashing of fields of young plant and ratoon canes in order to conserve soil moisture and keep down the temperature.

APPENDIX A.

PREPARATION OF MEDIA.

1. Sweet Potato Agar.

150 grms. of sweet potato sliced thin and cooked in 300 c.c. water for half an hour. The juice now filtered through a fine cloth and made up to 250 c.c. 10 grms. of powdered agar dissolved in 250 c.c. water and boiled for 5 minutes. To the potato juice cooled to 60°C . add 5 grms. egg albumen (white) previously mixed with 50 c.c. tap water. Now pour the melted agar into the potato juice slowly, stirring constantly. Autoclave at 120°C . for 20 minutes. Filter through cotton wool, tube and sterilize in autoclave at 120°C . for ten minutes.

2. Corn Meal Agar.

To 50 grms. of corn meal add 1 litre of water. Keep in a water bath for 1 hour at 58°C . (never over 60°C). Filter through paper. Add 1.25% agar flour. Steam for $1\frac{1}{2}$ hours. Filter and tube. Autoclave for 15 minutes at 115°C .

3 & 4. Lachnosterna and Diaprepes grub Agar.

Both prepared in the same manner. Dissolve 15 grms. agar in 500 c.c. water and boil for 5 minutes. Add 0.5% sodium chloride (5.0 grms) to the boiling agar. Prepare the grub infusion by taking about 200 grms fresh grubs and boiling with about 700 c.c. of tap water until a soup is formed. This grub infusion

is now titrated and if the acidity is more than +15° Fuller's scale then the reaction is adjusted to +15° Fuller's scale or some degree of less acidity to avoid liquification of agar. Now add to 500 c.c. of the grub infusion of known reaction and cooled to 60° C. 16 grms. of egg albumen (white) previously mixed with 100 c.c. tap water. Mix thoroughly. Pour the melted agar into the grub infusion slowly, stirring constantly. Autoclave at 120° C. for 20 minutes or more. Filter through absorbent cotton, tube and sterilize at 120° C. for 10 minutes.

5. *Cane Stem Infusion Agar.*

200 grms of sliced cane stem is boiled for 20 minutes in 500 c.c. water. To this infusion cooled to 60° C. 10 grms. of egg albumen mixed previously in 100 c.c. tap water is added and thoroughly mixed. 15 grms powdered agar are dissolved in 500 c.c. water, boiled for 5 minutes and added to the infusion stirring constantly. Further treatment is the same as in the case of Nos. 3 and 4.

6. *Ash Maltose Agar.*

A.	Wood Ashes	15 grms.
	Distilled water	500 c.c.

Solution A is now filtered and to every 400 c.c. of same 4 grms of maltose is added.

B.	Agar	12 grms.
	Distilled water	500 c.c.

Dissolve the agar and boil for 5 minutes. Pour solution B. into solution A. stirring constantly. Cool to 60° C. and add 10 grms. egg albumen dissolved in 100 c.c. tap water and stir thoroughly. Autoclave at 120° C. for 15 minutes, filter through absorbent cotton, tube and sterilize at 120° C. for 10 minutes.

7. *Soil Infusion Agar.*

A.	12½ grms of rich black soil.
	125 c.c. tap water.

Add water to soil in cold and allow to stand 30 minutes. Allow to settle and decant off the supernatant fluid.

B.	Water (tap)	125 c.c.
	Agar flour	3.2 grms.

Dissolve agar as usual and add solution B. to A. Cool to 60° C. and add 2.5 grms. egg albumen dissolved in 25 c.c. tap water, stir thoroughly, autoclave and treat exactly as in No. 6.

8. *Peptone Beef Agar.*

A.	Peptone (dry)	10 grms.
	Sodium chloride	5 grms.
	Beef Extract (Lemco)	5 grms.
	Water	500 c.c.

Boil three minutes and neutralize.

B.	Agar flour	12 grms.
	Water	500 c.c.

Pour melted B. into A. cool to 60° C. and add the whites of two eggs beaten in 50 c.c. water. Stir thoroughly and autoclave at 120° C. for 15 minutes. Filter through absorbent cotton, tube and sterilize at 120° C. for 10 minutes.

9. *Glucose Peptone Beef Agar.*

Same as in preparation of No. 8 but to solution A 2% glucose is added.

10. *Glycerine Peptone Beef Agar.*

Same as in preparation of No. 8 but to solution A 5% glycerine is added.

DESCRIPTION OF THE PROTOZOAN ORGANISM ASSOCIATED WITH THE BASAL STEM PORTIONS OF SUGAR CANE.

Active, non-encysted forms marginally ciliated, $19-30 \times 9.5-22$ microns. Hyaline to grey. Contents granular, often with a large vacuole. Somewhat kidney-shaped. Cysts hyaline to grey when first formed but orange yellow to brown with age, spherical and smooth; usually $13.5-16$ microns diameter, but may vary from $8-19$ microns diameter; contents granular and having somewhat thick hyaline walls. Yellowish or brown cysts have never been observed to germinate. Living cysts on germination break up into four actively motile ciliate protozoa within the thick walls which eventually become ruptured and liberate the motile organisms. Only found in the vascular system of the base of cane stems in some cases of true root disease. Grows well on sweet potato agar, sterilized portions of cane stem and sterile cane juice which have been inoculated with a mixed culture of these protozoa and bacteria.

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APPENDIX B.

PLATES
AND
EXPLANATIONS

EXPLANATION OF PLATE 1.

- FIG. 1. Photograph of portion of field of first ratoon canes (variety B.H. 10 (12)) suffering from root disease. Note the very stunted condition of the stool A in the centre and the wilted, shrivelled appearance of its leaves.
- FIG. 2. Photograph of portion of field of first ratoon canes (variety B.H. 10 (12)) suffering from root disease. Note the stunted condition of stools B and C when compared with stool D which apparently had not yet contracted the disease.

PLATE 1



Fig. 1



Fig. 2

EXPLANATION OF PLATE 2.

FIGS. 3 & 4. Photographs of pure cultures of *Rhizoctonia palida* = A and *Rhizoctonia solani* = B in culture flasks and tubes respectively. The medium in both flasks is sweet potato chips while that in the tube A is sweet potato agar and that in tube B is ash maltose agar. The latter fungus is seen almost filling the flask and producing abundant dark coloured, aerial mycelium and sclerotia in both flask and tube, while the former is not aerial in habit and produces light coloured mycelium and irregular yellowish non-aerial sclerotic masses as seen adhering to the walls of the tube toward the base.

PLATE 2.



Fig. 3

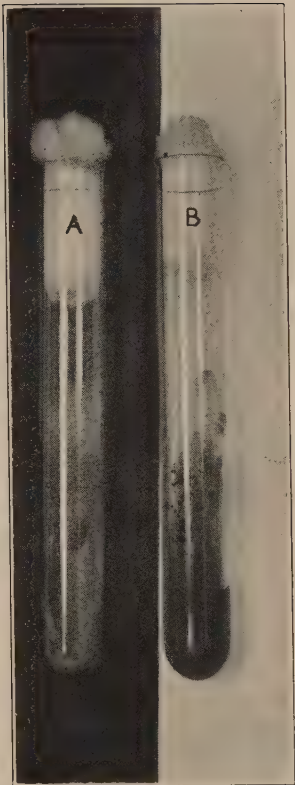


Fig. 4

EXPLANATION OF PLATE 3.

Figures 5, 6 and 7 are all photographs of the cane plants (variety Ba.6032) in the inoculation experiment with *Rhizoctonia solani*, Kuhn. Plants Nos. 2 and 3 were inoculated with pure cultures of the above fungus, while Nos. 4 and 5 are controls. Experiment started July 2, 1921.

FIG. 5. Photograph taken on August 27, 1921, fifty-six days subsequent to the planting of the four seed pieces. No visible difference in growth can be observed as yet between the inoculated and control plants.

FIG. 6. This picture was taken on March 9, 1922, just three weeks after all the plants in the tins had been cut off on Feb. 16, 1922. Prior to cutting off the canes the leaves of Nos. 2 and 3 were distinctly yellow compared with those of Nos. 4 and 5 which were normally green. As will be seen the rate of growth is distinctly in favour of both controls Nos. 4 and 5.

FIG. 7. This picture was taken on April 13, 1922 and shows the amount of growth which took place in the inoculated and control plants subsequent to the photo taken on March 9, (see fig. 6). The yellow appearance of the leaves and stunted condition of the inoculated plants Nos. 2 and 3 when compared with the normal green foliage and healthy growth of the controls Nos. 4 and 5 furnish ample proof of the ability of *Rhizoctonia solani* to reproduce the symptoms of root disease.

PLATE 3.



Fig. 5



Fig. 6



Fig. 7

EXPLANATION OF PLATE 4.

- FIG. 8. Photograph of root systems of the plants in plate 3, fig. 7. The photo was taken on April 21, 1922, eight days subsequent to the taking of the photograph in fig. 7. The control plants Nos. 4 and 5 are seen to have a greater amount of roots than the inoculated plants.
- FIG. 9. Photograph of root systems and stem portions of seedling canes (variety B. 60). No. 1 was inoculated with a pure culture of *Rhizoctonia solani* while No. 2 was left as control. The canes were grown in 8" pots and sterilized soil used to transplant the seedlings in. Equal conditions were given both plants which were originally of equal size and appearance. The photo was taken thirty-four weeks after the experiment was started and shows the difference in root system and amount of stem produced in favour of the control plant.
- FIG. 10. Photograph of the basal portion of stem and the adventitious roots of a typical case of root disease. a=the reddish areas on roots which are beginning to decay. b=the reddish diseased tissue of the basal part of the stem indicating that the fungus causing root decay has advanced into these tissues after attacking the roots. Most of the roots are seen to be blackened and dead.

PLATE 4.



Fig. 8



Fig. 9



Fig. 10

EXPLANATION OF PLATE 5.

Figures 11 and 12 are both photographs of the cane plants (variety Ba. 6032) in the inoculation experiment with *Rhizoctonia palida*. Plants Nos. 1 and 2 were inoculated with pure cultures of the above fungus while Nos. 3 and 4 are controls. Experiment started on February 15, 1922.

FIG. 11. Photograph taken on April 21, 1922, sixty-five days subsequent to the planting of the four seed pieces. No visible difference in growth can be observed as yet between the inoculated and control plants.

FIG. 12. Photograph taken on July 21, 1922, just ninety-one days after all the plants in the tins had been cut off on April 21. On June 7, the leaves of Nos. 1 and 2 were distinctly yellow compared with those of Nos. 3 and 4 which were normally green. As will be seen, the inoculated plants are distinctly stunted, particularly No. 1 when compared with the controls.

PLATE 5.

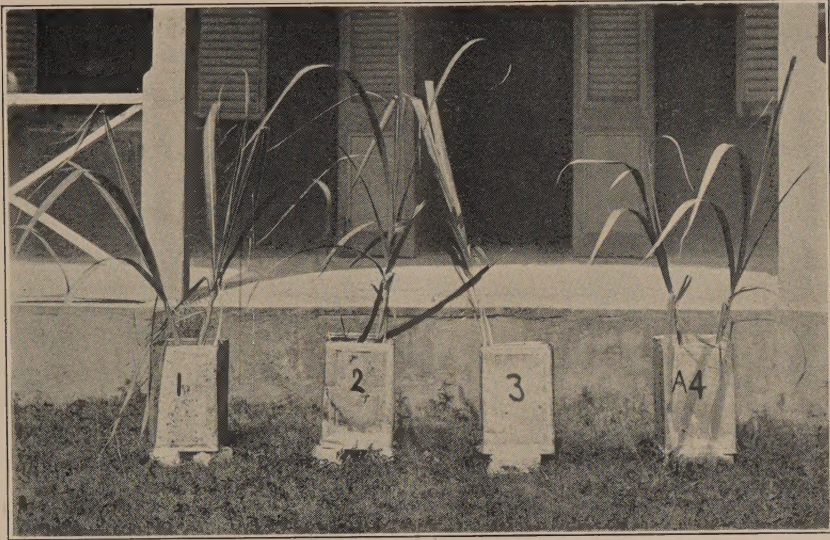


Fig. 11



Fig. 12

